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Research Article

Prevalence and Associated Risk Factors of Bovine Tuberculosis among Cattle (Domestic) and Buffaloes (Wild) Animals' Species in Asia: A Systematic Review and Meta-Analysis Protocol

Aboudou Habirou Kifouly[1-3]*, Orléanse Kouin[2], Géorcelin Alowanou[2], Obase Ngemani Bekindaka[1,3], Pierre Challaton[2], Abdou Satar Akadiri[5], John Dossou[2], Gilles-Christ Akakpo[2], and Cyrille Kodoéito Boko[4]

[1]*Pan African University Life and Earth Sciences Institute (Including Health and Agriculture), University of Ibadan, Nigeria*

[2]*Laboratoire d'Ethnopharmacologie et de Santé Animale, Faculté des Sciences Agronomiques, Université d'Abomey-Calavi, Bénin*

[3]*Immunology and Molecular Biology Laboratory of the Biotechnology Research Center, University of Yaoundé I, Yaoundé, Cameroon*

[4]*Unité de Recherche sur les Maladies Transmissibles, Ecole Polytechnique d'Abomey-Calavi, University d'Abomey-Calavi, Bénin*

[5]*Unité de Biochimie et Substances Naturelles Bioactives (UBSNB), Laboratoire de Biologie Intégrative et d'Innovation Thérapeutique (BioInov)*

***Corresponding author**

Aboudou Habirou Kifouly, Unité de Recherche sur les Maladies Transmissibles, Ecole Polytechnique d'Abomey-Calavi, University d'Abomey-Calavi, Bénin, Tel: (+229) 0151928481

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Abstract

Background: Bovine tuberculosis (bTB), caused by Mycobacterium bovis, is a chronic, debilitating zoonotic disease of major global importance, affecting both animals and humans, particularly in low- and middle-income countries especially in developing countries. Globally, bovine tuberculosis is widely distributed, with the highest prevalence reported in Asia and Africa, while Antarctica remains free of the disease.

Methods: A comprehensive and structured literature search will be conducted in accordance with the PRISMA 2020 guidelines. Relevant studies published between January 1990 and October 2025 will be systematically retrieved from multiple scientific databases, including PubMed, Google Scholar, Web of Science, Scopus, ScienceDirect, CAB Abstracts, SpringerLink, and African Journals Online [1], and additional articles will be also identified manually from reference lists of articles generated in database search, systemically for papers that presented bTB prevalence data, published in English and French language. A total of 62 published articles will be selected for inclusion in the meta-analysis.

Results: The analysis will be designed on the prevalence of bovine tuberculosis among animals' species and also will consider effect of various potential risk factors (age, weight, breed of different animals' species, herd size, animal status) along with different geographical factors associated with bTB prevalence, will be considered for consideration of their impact in the elaboration and strategies control programs of diverse animals' ailments especially bovine tuberculosis.

Conclusion: In conclusion, while this systematic review is expected to provide robust evidence on the bovine tuberculosis among animals' species, these limitations will be kept in mind when interpreting the results.

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BACKGROUND

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a chronic, debilitating zoonotic disease of major global importance, affecting both animals and humans, particularly in low- and middle-income countries. As a member of the *Mycobacterium tuberculosis* complex (MTC), bTB remains a significant constraint on public health, livestock productivity, and economic development due to its impact on animal health and its zoonotic potential, primarily through the consumption of infected or unpasteurized milk [2-4]. In humans, infection with *M. bovis* often leads to extrapulmonary tuberculosis, whereas in cattle it presents as a granulomatous, slowly progressive disease. Advanced clinical stages are characterized by emaciation, intermittent low-grade fever, enlargement of draining lymph nodes, and, in some cases, udder induration [5]. When the digestive tract is involved, clinical manifestations may include diarrhoea or constipation, as well as respiratory signs such as coughing and dyspnoea [6].

Globally, bovine tuberculosis is widely distributed, with the highest prevalence reported in Asia and Africa, while Antarctica remains free of the disease [7]. Between 2017 and 2018, approximately 44% of countries reported the presence of bTB; among these, 62% confirmed infections exclusively in livestock, whereas 35% reported infections in both livestock and wildlife [7]. It is estimated that more than 50 million cattle worldwide are affected by bTB, with the highest prevalence documented in India, where approximately 7.3% of farm and dairy cattle are infected [8]. In contrast, several countries—including Norway, Austria, Switzerland, Luxembourg, Canada, Sweden, Australia, and Japan—have successfully achieved or maintained bTB-free status through rigorous eradication programs [7].

Although *M. bovis* primarily infects cattle, it has been isolated from a wide range of domestic and wild species, including goats, sheep, pigs, buffaloes, deer, badgers, bison, camels, primates, dogs, and several endangered species [6-9]. Other members of the *Mycobacterium tuberculosis* complex, such as *M. caprae, M. orygis, M. microti*, and *M. africanum*, are also capable of infecting livestock and wildlife [5-10]. Transmission occurs predominantly through the inhalation of infectious aerosols generated by coughing or sneezing of infected animals, as well as through the ingestion of contaminated feed, water, or unpasteurized dairy products [2-9]. Vertical transmission is considered rare but may occur through infected milk or colostrum, or via in utero infection of calves [4]. In humans, zoonotic transmission mainly results from the consumption of raw or undercooked meat or from direct contact with infected animals and their biological fluids, particularly during handling and slaughter [11].

The prevalence of bTB varies considerably depending on diagnostic methods, animal populations, and geographic settings. Studies conducted in South Asia have reported heterogeneous prevalence estimates using tuberculin skin testing (TST) and molecular diagnostic techniques. In Bangladesh, TST positivity rates of 5.9% in Pabna and 3.05% in Mymensingh have been reported, while substantially higher rates were observed among breeding bulls at national research facilities, reaching 27.5% at the Central Cattle Breeding and Dairy Farm and 7.1% at the Bangladesh Livestock Research Institute [12,13]. Serological studies have further estimated seroprevalence rates of 7.5% in Chattogram, 5.9% in Mymensingh, and 7.8% in Sirajganj, highlighting the value of serological tools for elucidating disease dynamics and identifying associated risk factors [14-16]. A meta-analysis conducted by Taye et al. [17], demonstrated that the proportion of *M. bovis* among human tuberculosis cases ranged from 0.4% to 76.7%, with a pooled prevalence of 12.1%. In developing countries, *M. bovis* is estimated to account for 10–15% of new human TB cases, compared with only 1–2% in industrialized nations [4].

The movement of cattle from endemic regions constitutes a major risk factor for the spread of bTB [18], as infected animals may remain asymptomatic for prolonged periods. Crossbred cattle, Holstein–Friesian breeds, and indigenous cattle represent the main dairy populations in South Asia, where bTB remains one of the most important zoonotic diseases affecting both animal and human health [19-21].

From an economic perspective, bovine tuberculosis imposes substantial global losses, estimated to exceed USD 3 billion annually. These losses result from reduced milk production (10–18%), decreased productive efficiency (10–25%), condemnation of infected organs, reduced meat yield (approximately 15%), and increased mortality rates. Additional economic impacts arise from trade restrictions, compulsory culling programs, and limitations on animal movement [7]. Historical and molecular evidence indicates that humans and animals often share identical *M. bovis* strains, confirming interspecies “spillover” transmission [4-17]. Although many high-income countries have successfully controlled or eradicated bTB, sustaining a bTB-free status remains challenging due to the persistence of wildlife reservoirs and ongoing interspecies transmission [9].

Given the zoonotic and economic significance of *M. bovis*, a systematic review and meta-analysis are warranted to synthesize existing evidence on the seroprevalence, associated risk factors, and diagnostic approaches for bovine tuberculosis in Asia. Such an analysis will facilitate the identification of epidemiological trends, inform disease control and prevention strategies, and support the implementation of the One Health framework to reduce the burden of bTB in both animals and humans [2-21].

Overall, *Mycobacterium bovis* infection continues to pose a critical challenge at the human–animal interface, necessitating integrated One Health strategies that enhance surveillance systems, expand diagnostic coverage, and promote control measures tailored to the socio-economic contexts of endemic regions of Asia continent.

This systematic review and meta-analysis aim to synthesize available evidence to provide a comprehensive and consolidated estimate of the seroprevalence of bovine tuberculosis among cattle and other animal breeds included in studies conducted in Asia continent within the defined study period. In addition, the review seeks to identify the major risk factors associated with infection and to assess the diagnostic methods employed. The findings are expected to improve understanding of bTB epidemiology and to inform effective control strategies, thereby providing valuable insights for veterinary authorities, public health policymakers, and researchers engaged in zoonotic disease control and livestock health improvement.

MATERIALS AND METHODS

Information Sources and Search Strategy

A comprehensive and structured literature search will be conducted in accordance with the PRISMA 2020 guidelines [22]. Relevant studies published between January 1990 and October 2025 will be systematically retrieved from multiple scientific databases, including PubMed, Google Scholar, Web of Science [23], Scopus [24], ScienceDirect [25], CAB Abstracts [26], SpringerLink [27], and African Journals Online (AJOL) [1].

The search strategy employed Boolean operators and key terms as follows: ("Mycobacterium bovis" OR "bovine tuberculosis") AND ("seroprevalence" OR "risk factors" OR "detection methods") AND ("cattle" OR "animals' species") AND ("Asia"). Only peer-reviewed articles written in English or French will be considered eligible. Studies focusing simultaneously on multiple animal species diseases without providing bovine tuberculosis-specific data will be excluded. Additionally, the reference lists of selected articles will be carefully screened to identify further relevant publications that met the inclusion criteria.

Eligibility Criteria

Inclusion Criteria

Studies will be included in this review if they met the following criteria:

- The study must be a full-text article published in English or French.
- The study design will be cross-sectional, case-control, or cohort, reporting quantitative data on bovine tuberculosis (BTB) prevalence, risk factors, or diagnostic detection methods (e.g., tuberculin skin test, interferon-gamma assay, ELISA, PCR, or culture).
- The study population consisted exclusively of cattle and other animals' species sampled within Asia continent.
- The research provided clear numerical data (e.g., prevalence estimates, odds ratios, or confidence intervals) allowing inclusion in meta-analysis.
- Studies conducted between January 1990 and October 2025 will be considered, given that post-1990 publications reflect improved diagnostic and surveillance systems for bovine tuberculosis in the countries of Asia continent.

Exclusion Criteria

Studies will be excluded if they met any of the following conditions:

☐ They will be published outside the designated time frame (January 1990–October 2025).

☐ They involved species other than animals (ex: humans) or mixed animal populations without species-specific data.

☐ They addressed bovine tuberculosis without reporting direct evidence related to seroprevalence, risk factors, or detection methods.

☐ They will be reviews, conference abstracts, editorials, or unpublished theses lacking primary data.

Study Records

Data Management and Collection Process: All bibliographic records and retrieved data will be managed using Rayyan, a web-based systematic review tool designed to facilitate article screening, duplicate removal, and collaborative decision-making. Two independent reviewers imported the retrieved studies into Rayyan and classified them as "included," "excluded," or "undecided" based on predefined criteria. Extracted data included author names, publication year, study region, sample size, diagnostic method, prevalence rates, identified risk factors, and other relevant epidemiological information.

Selection Process: Article selection followed a two-step process. First, titles and abstracts will be independently screened by two reviewers to assess preliminary relevance. In the second phase, the full texts of potentially eligible articles will be retrieved and reviewed in detail. In the event of discrepancies between reviewers, a third reviewer adjudicated to reach a consensus on final inclusion.

PICO Framework: The inclusion and exclusion processes will be guided by the PICO (Population, Intervention, Comparison, Outcome) framework, as follows:

- Population (P):

Cattle and other animals' species of any breed, age, or sex, reared in various ecological and administrative regions of Asia, will be included.

- Intervention (I):

Any intervention on prevalence rate, age or animal' breed or diagnostic approach aimed at identifying Mycobacterium bovis infection, including tuberculin skin tests (TST), ELISA, PCR, culture, and interferon-gamma assays and many others diagnostic methods.

- Comparison (C):

Comparative analyses will be based on geographical regions, production systems, objective of animals raising, specimen or diagnostic methods used.

- Outcomes (O):

The mainly outcomes will be the seroprevalence of bovine tuberculosis and the identification of risk factors such as age, breed, sex, herd size, management system, and contact with wildlife.

Data Extraction

Study Selection Procedure: Titles and/or abstracts of potentially relevant studies will be retrieved through the predefined search strategy, supplemented by additional manual searches from reference lists and grey literature. Two independent reviewers screened all titles and abstracts to identify studies that appeared to meet the inclusion criteria. Full-text versions of these potentially eligible articles will be subsequently obtained and independently assessed by two members of the review team for final eligibility.

- Any discrepancies regarding study inclusion will be resolved through discussion, and when necessary, by consulting a third reviewer to reach consensus.
- A standardized, pre-tested data extraction form will be employed to ensure methodological consistency and to facilitate the assessment of study quality and evidence synthesis. Extracted information included Country and year of publication, study design and sampling strategy, animal species and sample size, number of cattle testing positive for bovine tuberculosis, diagnostic methods employed, type of specimens collected, results obtained, and information relevant to risk of bias and methodological quality.
- When critical data will be missing or unclear, the corresponding authors will be contacted to obtain the necessary information.

Data Selection and Extraction Methods: Two reviewers independently extracted data from each included study using the standardized extraction template. Numerical data will be primarily retrieved from tables, figures, or textual descriptions. In instances where data will be presented graphically without numerical reporting, extraction will be performed using digital data extraction software.

When essential data will be unavailable or ambiguous, the reviewers attempted to contact the corresponding authors via email (up to two attempts). If an outcome (e.g., bovine tuberculosis prevalence or diagnostic test results) will be measured at multiple time points, the data corresponding to the time point with the highest reported efficacy or relevance will be selected for inclusion in the synthesis.

Quality Assessment and Risk of Bias Evaluation: All included studies will be rigorously assessed for methodological quality, risk of bias, and overall reliability of the evidence. To ensure robustness, three complementary quality assessment tools will be employed:

- SYRCLE's Risk of Bias Tool – specifically developed for animal research [28];

- The CAMARADES Checklist – assessing experimental rigor and transparency [29]; and
- The Joanna Briggs Institute (JBI) Critical Appraisal Checklist for Prevalence Studies – evaluating epidemiological soundness [30].

The integration of these three instruments will be justified by the mixed methodological nature of the included studies: SYRCLE provided insights into experimental validity, CAMARADES enhanced the appraisal of study design rigor, and JBI ensured accuracy in prevalence estimation.

Each study will be independently evaluated by two reviewers, with disagreements resolved through consensus or arbitration by a third reviewer. A total quality score exceeding 70% will be interpreted as indicative of high-quality evidence.

- For SYRCLE's tool, the following domains will be evaluated: (1) generation and concealment of the allocation sequence; (2) baseline comparability of groups or adjustment for confounders; (3) randomization of housing; (4) blinding of caregivers and investigators; (5) random selection for outcome assessment; and (6) blinding of outcome assessors.
- The CAMARADES checklist further considered criteria such as peer-review status, inclusion of control groups, random treatment allocation, blinded induction and assessment, clarity of animal models, reporting of statistical methods, compliance with animal welfare standards, and declaration of conflicts of interest.
- Finally, the JBI checklist will be applied to ensure the methodological soundness of studies reporting prevalence data, strengthening the epidemiological relevance of the synthesis.
- All screening and data management processes will be performed using EndNote X9 for reference organization and Rayyan software [31] for systematic screening, ensuring transparency, reproducibility, and auditability.

Statistical Analysis

Data synthesis will be performed using both narrative and quantitative (meta-analytic) approaches. Initially, a structured narrative synthesis summarized study characteristics, interventions, diagnostic methods, and target populations. Quantitative analyses will be then performed using appropriate statistical techniques. For dichotomous outcomes, intervention effects will be expressed as risk ratios (RRs), while continuous outcomes will be summarized using standardized mean differences (SMDs) to allow comparability across diverse studies. When continuous data will be reported in different measurement units, standardization procedures will be applied to ensure uniformity. In cases where numerical data will be incomplete, corresponding authors will be contacted. If data retrieval will be unsuccessful, standard imputation techniques—such as deriving standard deviations from reported standard errors, confidence intervals, or p-values—will be employed. Studies with insufficient data for imputation will be excluded from the quantitative synthesis but retained for qualitative discussion. To account for inter-study heterogeneity, a random-effects model will be applied, with results presented as pooled estimates and 95% confidence intervals (CIs). Statistical heterogeneity will be evaluated using the Chi-square test and quantified by the I^2 statistic; values above 50% indicated substantial heterogeneity. All analyses will be conducted using Review Manager (RevMan) version 5.4.

Exploratory analyses—including subgroup analyses and sensitivity testing—will be conducted based on study quality, diagnostic method, geographic region, and animal species. Sensitivity analyses excluding low-quality studies will be performed to assess the robustness of pooled estimates. Potential publication bias will be assessed using visual inspection of funnel plots and further evaluated with Egger's regression test [32-57], ensuring transparency and minimizing interpretative bias.

ETHICAL CONSIDERATIONS AND DISSEMINATION

This systematic review and meta-analysis relied solely on data derived from previously published studies; therefore, no new ethical approval will be required. Nevertheless, during data extraction, the review team verified that all included studies had received appropriate ethical clearance from institutional or national committees. The review protocol will be registered in the International Prospective Register of Systematic Reviews (PROSPERO) to enhance transparency, prevent duplication, and uphold methodological rigor.

The findings of this review will be disseminated through publication in a peer-reviewed scientific journal, presentation at international conferences, and communication with global health and livestock development organizations. These efforts aim to inform evidence-based policy formulation, strengthen bovine tuberculosis surveillance, and guide control strategies in Asia and beyond.

Timeline

Activity	Duration
Protocol registration (PROSPERO)	2 weeks
Literature search	3 weeks
Screening and selection	2 weeks
Data extraction and quality appraisal	3 weeks
Data analysis and interpretation	3 weeks
Manuscript writing and submission	3 weeks

EXPECTED OUTCOMES

– The analysis will be designed on the prevalence of bovine tuberculosis among animals' species and also will consider effect of various potential risk factors (age, weight, breed of different animals' species, herd size, animal status) along with different geographical factors associated with bTB prevalence, will be considered for consideration of their impact in the elaboration and strategies control programs of diverse animals' ailments especially bovine tuberculosis.

CONCLUSION

In conclusion, while this systematic review is expected to provide robust evidence on the bovine tuberculosis among animals' species, these limitations will be kept in mind when interpreting the results. Strategies such as subgroup analysis, sensitivity analysis, and inclusion of diverse sources of data will be employed to minimize the impact of these limitations and enhance the reliability of conclusions drawn from the evidence.

LIMITATIONS

- Potential publication bias.
- Language restriction to English and French.
- Variability in tuberculosis symptoms effects and study quality.
- Heterogeneity in animals' species and diagnostic methods.
- Limited standardization of outcome measures.

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